Exhibit A

CIE INVENTION REPORT AND RECORD FORM

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DESCRIPTIVE TITLE OF INVENTION

Novel anti-arrhythmic drugs that target the leak in the ryanodine receptor (RyR2)____

____

GRANT/CONTACT (If any):

Sponsor(s) ______________________________ Award Number __________________

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We have shown that the drug JTV-519 (aka K201) fixes the leak in the ryanodine recepotr/calcium release channel that triggers sudden cardiac death due to fatal cardiac arrhythmias. [Redacted information.]

A.

1) RyR2 is a target for preventing cardiac arrhythmias that cause sudden cardiac death. RyR2 is the major calcium release channel in the heart required for cardiac muscle excitation-contraction coupling. Mutations in RyR2 are linked to a rare genetic disorder called catecholaminergic polymiorphic ventricular tachycardia (CPVT). Patients with CPVT have exercise induced VT that can cause sudden cardiac death. We made mutant RyR2 with seven different CPVT mutations and studied their function. All seven have functional defects that result in channels that become leaky when stimulated during exercise. Since the channels on on the sarcoplasmic reticulum (SR) the leak is referred to as an SR calcium leak. SR calcium leak has been known to be a trigger for VT because SR calcium leak cause delayed after depolarizations (DADs) that trigger VT. Our study is the first to identify a mechanism the SR calcium leak that causes DADs. Remarkably the defect in the mutant CPVT channels makes them look like the leaky channels in the hearts of patients with end stage heart failure – a disorder characterized by a high incidence of fatal cardiac arrhythmias. Therefore, we propose that the mechanism for the VT in CPVT is the same as the mechanism for VT in heart failure. Since heart failure is a leading cause of death world wide – methods to fix the leak in RyR2 could prevent fatal arrhythmias in millions of patients world-wide.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disorder in individuals with structurally normal hearts, characterized by stress-induced ventricular tachycardia, a lethal arrhythmia that may cause sudden cardiac death. Mutations in the cardiac calcium (Ca^{2+}) release channel/ryanodine receptor type 2 (RyR2), located on the sarcoplasmic reticulum (SR) and required for excitation-contraction (EC) coupling in the heart, have been linked to CPVT. To determine the molecular mechanism underlying the fatal cardiac arrhythmias in CPVT we studied four CPVT-associated mutant RyR2 channels (S2246L, R2474S, N4104K, R4497C). All individuals with CPVT have exercise-induced cardiac arrhythmias. We now show that exercise activates RyR2 due to phosphorylation by adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA). Mutant RyR2, that had normal function in planar lipid bilayers under basal conditions, were more sensitive to activation by PKA phosphorylation exhibiting increased activity (open probability) and prolonged open states compared to wild type channels. In addition, PKA phosphorylated mutant RyR2 channels were resistant to inhibition by Mg^{2+}, a physiological inhibitor of the channel, and showed reduced binding to FKBP12.6, a protein that stabilizes the channel in the closed state. These findings suggest that during exercise, when the RyR2 are PKA phosphorylated, the mutant CPVT channels are more likely to open in the relaxation phase of the cardiac cycle (diastole), increasing the likelihood of arrhythmias triggered by SR Ca^{2+} leak.

2) Assays for Activation of the Cardiac Rynaodine Receptor/Calcium Release Channel (RyR2)

Our data show that protein kinase A (PKA) phosphorylation of the cardiac rynaodine receptor/calcium release channel (RyR2) on serine 2809 activates the channel by releasing the FK506 binding protein 12.6 (FKBP12.6). (These data are in press in Cell.) In failing hearts (human as well as animal models of heart failure) RyR2 is PKA hyperphosphorylated resulting in defective channels that have decreased amounts of FKBP12.6 bound to them and have increased sensitivity to calcium-induced activation. The net result of these changes is that the RyR2 channels are "leaky". These "leaky" channels can result in depletion of intracellular stores of calcium such that there is not enough calcium in the sarcoplasmic reticulum to provide a strong stimulus for muscle contraction. This results in weak contraction of heart muscle. A second consequence of the "leaky" RyR2 channels is that they release calcium during the resting phase of the heart cycle known as "diastole". This release of calcium during diastole can trigger fatal arrhythmias of the heart (eg. Ventricular tachycardia and ventricular fibrillation) that cause sudden cardiac death.

Our data also show that treatment of heart failure with a mechanical pumping device referred to as a Left Ventricular Assist Device (LVAD) which puts the heart at rest and restores normalized function is associated with a reduction in the PKA hyperphosphorylation of RyR2 and normalized function of the channel.

We also have new published data showing that treatment of dogs (with pacing induced heart failure) with beta adrenergic blockers (beta blockers) reverses the PKA hyperphosphorylation of RyR2. Beta blockers inhibit the pathway that activates PKA and have been used successfully to treat patients with heart failure, although prior to our study there has been no real understanding of why or how beta blockers are beneficial to heart failure patients.

One result from our work is that PKA phosphorylation of RyR2 increases the activity of the channel resulting in the release of more calcium into the cell for a given trigger (activator) of the channel. Compounds that block PKA activation would be expected to reduce the activation of the RyR2 channel resulting in less release of calcium into the cell. Compounds that bind to the RyR2 channel at the FKBP12 binding site but do not come off the channel when the channel is phosphorylated by PKA would also be expected to decrease the activity of the channel in response to PKA activation or other triggers that activate the RyR2 channel. Such compounds would also result in less calcium release into the cell.

1. Diagnostic assays

a) Assay the release of calcium into cells via the RyR2 channel using calcium-sensitive fluorescent dyes (eg. Fluo-3, Fura-2 and others). This assay involves loading cells with the fluorescent dye and stimulating the cells with RyR2 activators and determining whether or not compounds added to the cell reduce the calcium-dependent fluorescent signal [1-3]. Calcium dependent fluorescent signals are monitored with a photomultiplier tube and analyzed with appropriate software as described [1-3]. This assay can be easily automated to screen large numbers of compounds using multiwell dishes. This assay would be designed to identify compounds that inhibit the PKA dependent activation of RyR2-mediated intracellular calcium release. The assay involves expressing recombinant RyR2 channels in heterlogous expression system such as Sf9, HEK293 or CHO cells [1]. RyR2 could be co-expressed with beta adrenergic receptors as well. This would permit the assessment of compounds on RyR2 activation in response to addition of beta adrenergic receptor agonists.

b) Measure the level of PKA phosphorylation of RyR2 which correlates with the degree of heart failure and can be used to determine the efficacy of compounds designed to block the PKA phosphorylation of the RyR2 channel. This assay is based on the use of antibodies that are specific for the RyR2 channel protein. For this assay the RyR2 channel protein is immunoprecipitated and then back-phosphorylated with PKA and [γ^{32}P]-ATP. The amount of radioactive ^{32}P label that is transferred to the RyR2 protein can be measured using a phosphorimager [4].

References:

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2. Gillo, B., Ma, Y.S. & Marks, A.R. Calcium Entry During Induced Differentiation in Murine Erythroleukemia Cells. *Blood* **81**, 783-792 (1993).
3. Jayaraman, T., Ondrias, K., Ondriasova, E. & Marks, A.R. Regulation of the Inositol 1,4,5-Trisphosphate Receptor by Tyrosine Phosphorylation. *Science* **272**, 1492-1494 (1996).
4. Marx, S.O. *et al.* PKA Phosphorylation Dissociates FKBP12.6 from the Calcium Release Channel (Ryanodine Receptor): Defective Regulation in Failing Hearts. *Cell* **in press**(2000).

3) **[Redacted information]**

4) **[Redacted information]**

5) **[Redacted information]**

6) **[Redacted information]**

7) **[Redacted information]**

8) THIS APPLICATION FOCUSES ON JTV-519 (K201) A MEMBER OF THE 1,4 BENZODIAXEPINE FAMILY OF COMPOUNDS – OUT UNPUBLISHED DATA SHOW THAT JTV-519 ENHANCES BINDING OFG FKBP12.6 TO PKA PHOSPHORYLATED RYR2 AND TO MUTANT RYR2 THAT OTHERWISE HAVE REDUCED AFFINITY OR DO NOT BIND ANY FKBP12.6. THIS ACTION OF JTV-519 FIXES THE LEAK IN RYR2 **THAT TRIGGERS FATAL CARDIAC ARRYTHMIAS** AND CONTRIBUTES TO HEART MUSCLE DYSFUNCTION IN **HEART FAILURE**